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Full Length Research Paper

Biodiesel production from marine microalgae *Chlorella marina* and *Nannochloropsis salina*

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The aim of the study was to obtain high quality biodiesel from microalgae *Chlorella marina* and *Nannochloropsis salina* through transesterification. Growth studies revealed that maximum cell growth rate was obtained at 15th day of the culture. The flocculation activity result showed that pH 11 was optimum for cell flocculation at 37°C. In the present study, 60.26% of biodiesel yielded from 0.752 g L^{-1} contains 30% oil content from *N. salina*, whereas 50% yielded from 0.527 g L^{-1} contains 20% oil content. The crude lipid content found in *C. marina* and *N. salina* was found to be 20.33 ± 1.82% and 32.13 ± 1.40% of dry biomass. The density and viscosity of the biodiesel obtained from the crude lipid of *N. salina* and *C. marina* were 0.992 and 0.971 (kg L^{-1}), viscosity 3.2 and 4.8 (Pa·sat 40°C), respectively. The method implemented in this study could be novel approach and great potential in the industrial production of liquid fuel from microalgae.

Key words: Microalgae, *Nannochloropsis salina*, *Chlorella marina*, lipids, biodiesel, flocculation.

INTRODUCTION

Mechanical energy cannot be achieved successfully without petroleum, natural gas, coal, hydro electricity and nuclear energy; and they became the basic natural sources for the energy. The demand of petroleum and its by-products are increasing continuously due to the increase in population and industrialization. The discriminate use of petroleum sourced fuels is now widely recognized as unsustainable because it is non-renewable resources. In the last 10 years, many studies have been conducted on biofuels for substituting fossil fuels and reduce the greenhouse gas (GHG) emission which is responsible for global warming (Bastianoni et al., 2008).

Biodiesel production from microalgae is an emerging technology considered by many as a very promising source of energy, mainly because of its reduced competition for land. Among these, especially, microalgae were found to be an alternative nature source of renewable petroleum resources that is capable of meeting the global demand for fuels (Chisti, 2007, 2008). The idea of using algae as a source of fuel is not new (Chisti, 1981; Nagle and Lemke, 1990; Sawayama et al., 1995), but it is now being taken seriously because of the increasing price of petroleum and more significantly, the emerging concern about global warm that is associated with burning fossil fuels (Gavrilescu and Chisti, 2005). It is reported that microalgae can provide several different types of renewable biofuels which include, methane, biodiesel and biohydrogen (Gavrilescu and Chisti, 2005; Kapdan and Kargi, 2006; Spolaore et al., 2006).

Microalgae have short life cycle and use a photosynthetic process similar to higher plants for their energy. In fact, the biomass doubling time for microalgae during exponential growth is found as short as 3.5 h. Microalgae are veritable miniature biochemical factories, and appear photosynthetically more efficient than terrestrial plant, and are efficient CO_2 fixer (Pirt, 1986). The ability of algae to fix CO_2 has been proposed as a method of removing CO_2 from fuel gases from power plants, and thus, can be used to reduce emission of GHG (Chisti, 2007). Many algae are exceedingly rich in oil, which can be converted to biodiesel. The oil content of some

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microalgae exceeds 80% of dry weight (DW) of algae biomass (Banerjee et al., 2002; Chisti, 2007). Microalgae are faster in growth in the marine environment and yield of oil from algae is estimated between 5000 to 20000 m^3 / 4046 m^2/yr which is 7 to 31 times greater than the terrestrial crop, palm oil (635 m^3) (Pringsheim, 1950). The high growth rate of microalgae makes it possible to satisfy the massive demand on biofuels using limited land resources. Microalgae cultivation consumes less water than land crops. Most microalgae biomass contains three main components such as 1) lipids, 2) proteins, and 3) carbohydrates and/or hydrocarbons. Microalgae produce and store lipids in the form of fatty acids, phospholipids, glycolipids and it can be used as feedstocks for biodiesel production by transesterification reaction in the presence of acid or base with methanol. Hence, in the present study, the growth, flocculation rate, oil content and biodiesel production from *Nannochloropsis salina* and *Chlorella marina* was investigated.

MATERIALS AND METHODS

Culture collection and growth condition

Based on the availability and culture acclimatization, two microalgae such as *N. salina*, and *C. marina* were obtained from Rajiv Gandhi Centre for Aquaculture, Sirkali, Tamilnadu, India and used for the recovery of biodiesel. The cultures were grown in filtered and autoclaved seawater (30 psu, 8.0 pH) using Conway medium (Walne, 1966). The sterilized medium was kept for 2 days before inoculating microalgae for CO_2 equilibration. For culture of microalgae, 10 L of medium was placed in 15 L plastic container incubated for 15 days at 25 ± 1°C with aeration through mechanical stirrer and 12 h photoperiod by artificial light.

Algal growth rate

The biomass of the cultures was estimated for every 24 h by measuring the optical density at wavelength of 680 nm (Huang et al., 2002). Cells were harvested at late logarithmic phase and used for further experiments. To estimate DW, cultures were filter through 0.22 µm membrane filter, washed three times with distilled water to removed NaCl and dried the filters at 100°C for 4 h. The specific growth rate was calculated by the slope of logarithmic phase in terms of biomass of cells.

Determination of flocculation activity

The algal culture suspension (50 ml) of *N. salina* and *C. marina* were placed in a different 100 ml beaker. The pH was adjusted to 9 to 12 with HCl or NaOH and reference samples were also prepared without flocculants. The beakers were stirred at 100 rpm for 1 min at room temperature and left for 10 min to settle. After the flocculation of algal cells, an aliquot of culture was withdrawn at a level two-third from the bottom of the beaker and the absorbance measured at 680 nm (Shimadzu, spectrophotometer). Flocculation activity was calculated by the following equation (Toeda and Kurane, 1991):

Flocculation activity = [(1/A) – (1/B)],

A = absorbance of sample; B = absorbance of reference sample.

Dewatering of harvested microalgae

For dewatering of cells, harvested wet cell mass was frozen over night at -70°C and freeze dried (Grima et al., 1994).

Lipid extraction and estimation

The total lipids from the harvested cell mass were extracted three times by mixing chloroform-methanol (1:1 v/v) with a proportion of 1:1 using a slightly modified version method by Bligh and Dyer (1959). The mixtures were transferred into a separatory funnel and shaken for 5 min. The lipid fraction was then separated from the separatory funnel and the solvent evaporated using a rotary evaporator. The weight of the crude lipid obtained from each sample was measured using an electronic scale.

Transesterification and biodiesel production

The greenish extracted oil was evaporated to release the solvent mixture using rotary evaporator at 40 to 45°C. Then, the oil produced from algal species was mixed with a mixture of oil: NaOH: methanol (1: 5: 1) with stirring properly for 20 min. The mixture was kept for 3 h in electric shaker at 3000 rpm and kept for 16 h at 37°C to settle the biodiesel and the sediment layers clearly. The biodiesel layer was separated from sedimentation by flask separator. Biodiesel was washed by 5% water many times until it becomes clear; then biodiesel was dried by using dryer and finally kept under the running fan for 12 h. The produced biodiesel from 10 g wet mass of microalgae was measured using measuring cylinder and stored for further analysis.

Analytical methods

The properties of biodiesel such as density, viscosity, flash point, solidifying point cold filter plugging point, acid value, heating value, and hydrogen to carbon (H/C) ratio were measured. A comparison of these properties was made with biodiesel from micro algal oil and ASTM biodiesel standards (Antolin et al., 2002).

RESULTS AND DISCUSSION

In the present investigation, *N. salina* and *C. marina* have been chosen as species mainly because they are significant in this study; lead to quantitative estimates of growth rate and flocculation activity, cell biomass productivities and their lipid content enhance biodiesel productivity. These two microalgae species were incubated for 15 days for biomass production. The growth curve (Figure 1) of *N. salina* and *C. marina* was determined from parallel cultures starting from inoculums. Cell growth was started from the 1st day itself and it reaches maximum at 15th day of the culture. The growth rate of *N. salina* (0.752 g L^{-1}) was higher compared with *C. marina* (0.527 g L^{-1}) whose similar results were observed in the culture of *Botryococcus braunii* (Lee et al., 1998).

The flocculent (NaOH) concentration is a critical parameter for flocculation processes; it influences both extent and rate flocculation. Hence, flocculation activity experiments were undertaken to determine the effect of

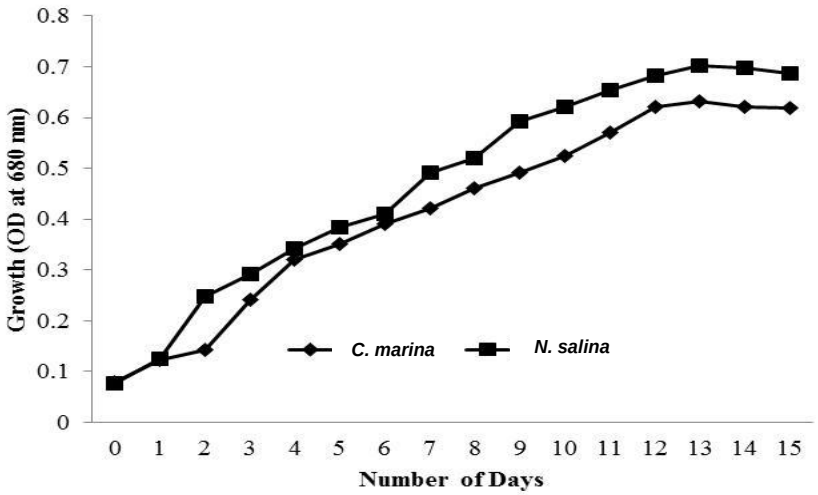

Figure 1. Growth rate microalgae *C. marina* and *N. salina*.

pH and optimum flocculent concentration on the flocculation of algal cells cultured for 1 week. The flocculation efficiency of *N. salina* and *C. marina* cultures were increased while increasing pH from 9 to 12. During the initial stage of flocculation process, when the pH of medium was increased, the small particles aggregated and slowly settled due to gravitational force. The cells formed large loose and dense packed aggregates that settled under gravitational force. Once the fine capture achieved equilibrium, further addition of flocculants might lead to the formation of larger aggregates. This in turn might cause higher settling rates with minimal addition of flocculants (Owen et al., 2002).

Results from this study revealed that flocculation activity of *N. salina* and *C. marina* was higher at pH 11. Similarly, the microalgae *B. braunii* exhibited maximum flocculation activity at pH 11 (Lee et al., 1998). These results (Figure 2) suggest that pH adjustment of the culture solution after 1 week is the most effective recovery of lipid. The most effective harvesting process for the recovery of *B. braunii* is pH adjustment (pH 11) after 2 weeks of incubation and higher pH levels were effective in algal sedimentation (Buelna et al., 1990).

The extracted greenish oil content (% oil/g of dry algal biomass) from harvested algae was (Table 1) determined by measuring weights of extracted oil and it was found to be 32.13 and 20.33% of biomass of *N. salina* and *C. marina* respectively. The biomass concentration and oil content of *Nanochloropsis* sp. (Chisti, 2007) and *Neochloris oleabundans* (Tornabene et al., 1983) were similar to *N. salina* and *C. marina*. In the present study, the higher yield of biodiesel was obtained in the *N. salina* and *C. marina*. Moreover, 60.26% of biodiesel yielded from 0.752 g L^{-1} contains 30% oil content from *N. salina* and 50% of yield from 0.527 g L^{-1} contains 20% oil content, whereas, 55.3% of biodiesel was achieved from *Chlorella protothecoides* after 144 h (Wu et al., 1994).

The characters of biodiesel obtained from *N. salina* and *C. marina* are shown in Table 2. The properties of biodiesel from *N. salina* were; density 0.992 (kg L^{-1}), viscosity 3.2 (Pa·sat 40°C), heating value 40 (MJ kg^{-1}) and H/C ratio 1:82, whereas the properties of *C. marina* were; density 0.971 (kg L^{-1}), viscosity 4.8 (Pa·sat 40°C), heating value 43 (MJ kg^{-1}) and H/C ratio 1:80. The biodiesel which was obtained from heterotrophic microalgae *C. protothecoides* was characterized by a high heating value of 41 MJ kg^{-1}, density of 0.864 kg L^{-1}, and a viscosity of 5.2×10^{-4} Pa·s (40°C) (Wu et al., 1994). Thus, the physical and fuel properties of biodiesel from the both microalgae were comparable to those of diesel fuel. The biodiesel from micro algal oil showed much lower cold filter plugging point of -10°C which clearly indicated the high quality of the biodiesel. The results suggest that the new process was a low-cost, feasible, and effective method for the production of high quality biodiesel from microalgae.

Conclusion

Compared with terrestrial crops which take a season to grow and only contain a maximum of about 5% DW of oil-microalgae, grow quickly and contain high oil content. This is why microalgae are the focus in the algae-to-biofuel arena. Oil content of microalgae is usually between 20 and 50%, while some strains can reach as high as 80%. Hence, the present study was made on culture of two different microalgae, growth, flocculation activities, oil content and identification by using ASTM standards. The results obtained from this investigation revealed that *N. salina* and *C. marina* were easy to cultivate which contains high lipid content. The faster growth rate as well as higher oil content found with these microalgae will make these as the potential candidate for

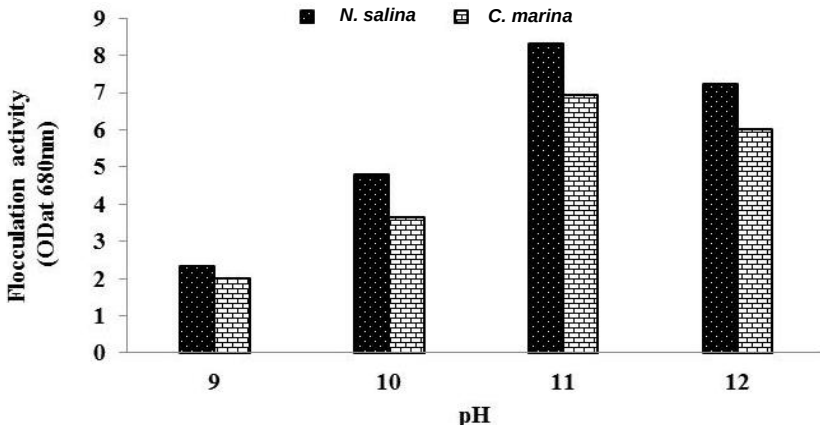

Figure 2. Effects of pH values and flocculants concentration on the flocculation of microalgae *C. marina* and *N. salina*.

Table 1. Cell biomass and lipid content of microalgae.

Microalgae	Biomass (g/L)	Lipid content (%)	Biodiesel yield (%)
N. salina	0.752 ± 0.012	32.13 ± 1.40	60.26 ± 2.11
C. marina	0.527 ± 0.012	20.33 ± 1.82	50.00 ± 1.80

The results were presented as mean ± SD. n = 3.

Table 2. Comparison of biodiesel from microalgae oil and diesel and ASTM biodiesel.

Property	*N. salina* biodiesel	*C. marina* biodiesel	ASTM biodiesel standard
Density (kg L^{-1})	0.992	0.971	0.86 - 0.90
Viscosity (Pa·sat 40°C)	3.2	4.8	3.5 - 5.0
Flash point (°C)	95	98	Minimum 100
Solidifying point (°C)	-4	-4	-
Cold filter plugging point (°C)	-10	-10	Summer maximum 0
Acid value (mg KOH g^{-1})	0.32	0.474	Max 0.5
Heating value (MJ kg^{-1})	40	43	-
H/C ratio	1.82	1.80	-

alternative biodiesel production.

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